Mechanisms of persistence, innate immune activation and immunomodulation by the gastric pathogen Helicobacter pylori

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Abstract: The gastric bacterium Helicobacter pylori efficiently evades innate immune detection and persistently colonizes its human host. Understanding the genetic determinants that H. pylori uses to establish and maintain persistence, along with their cellular targets, is key to our understanding of the pathogenesis of this extraordinarily successful bacterial colonizer of the human stomach. This review highlights recent advances in elucidating innate immune recognition of H. pylori, its interactions with myeloid cells and the consequences that this very local infection has for immune responses at extragastric sites in models of allergy, autoimmunity and parasitic infection. The human-specific, gram-negative gastric colonizer and carcinogen H. pylori represents the prototype of a persistent bacterial pathogen. It is transmitted during early childhood, typically from mother to infant, and is believed to persist in its human host from the cradle to the grave. The tremendous success of H. pylori in infecting and colonizing half of the world's population, and in continuously accompanying humans since they migrated out of Africa over 60000 years ago, can largely be attributed to its ability to manipulate the host immune system to its own advantage, and to thereby ensure its own persistence and chronicity. In his final years as an active PI, Stanley Falkow increasingly recognized the need to understand bacterial persistence strategies as a prerequisite of understanding the pathogenesis of chronic bacterial infections, and, inspired in large part by Denise Monack's work on Salmonella persistence, many of our discussions at the time revolved around this topic. Multiple labs have since made important contributions to our understanding of innate immune detection of H. pylori, the types and polarization of adaptive immune responses that ensue, the ability of H. pylori to skew such immune responses to its advantage, and its ability to manipulate the host immune system with far-reaching, even systemic consequences. This review attempts to cover some of these topics, with a particular focus on the most recent contributions by researchers in the field.

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Mechanisms of persistence, innate immune activation and immunomodulation by the gastric pathogen *Helicobacter pylori*

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Declaration of interest: none

Abstract

The gastric bacterium *Helicobacter pylori* efficiently evades innate immune detection and persistently colonizes its human host. Understanding the genetic determinants that *H. pylori* uses to establish and maintain persistence, along with their cellular targets, is key to our understanding of the pathogenesis of this extraordinarily successful bacterial colonizer of the human stomach. This review highlights recent advances in elucidating innate immune recognition of *H. pylori*, its interactions with myeloid cells and the consequences that this very local infection has for immune responses at extragastric sites in models of allergy, autoimmunity and parasitic infection.

The human-specific, gram-negative gastric colonizer and carcinogen *Helicobacter pylori* represents the prototype of a persistent bacterial pathogen. It is transmitted during early childhood, typically from mother to infant, and is believed to persist in its human host from the cradle to the grave. The tremendous success of *H. pylori* in infecting and colonizing half of the world's population, and in continuously accompanying humans since they migrated out of Africa over 60.000 years ago, can largely be attributed to its ability to manipulate the host immune system to its own advantage, and to thereby ensure its own persistence and chronicity. In his final years as an active PI, Stanley Falkow increasingly recognized the need to understand bacterial persistence strategies as a prerequisite of understanding the pathogenesis of chronic bacterial infections, and, inspired in large part by Denise Monack's work on *Salmonella* persistence, many of our discussions at the time revolved around this topic. Multiple labs have since made important contributions to our understanding of innate immune detection of *H. pylori*, the types and polarization of adaptive immune responses that ensue, the ability of *H. pylori* to skew such immune responses to its advantage, and its ability to manipulate the host immune system with far-reaching, even systemic consequences. This review attempts to cover some of these topics, with a particular focus on the most recent contributions by researchers in the field.

Innate immune recognition of *H. pylori* LPS intermediates via the TIFA/ALPK1 axis

H. pylori has long been known to activate canonical NF-κB signalling in gastric epithelial cells through mechanisms that depend on the bacterium's Cag pathogenicity island-encoded type IV secretion system (T4SS) [1]. NF-κB is a central transcriptional regulator of innate immune responses, controlling the expression of chemokines and cytokines involved in immune cell recruitment, as well as epithelial cell-intrinsic innate defense mechanisms. NF-κB signalling is activated directly by microbial pathogen-associated molecular patterns (PAMPs) or by pro-inflammatory cytokines such as TNF-α [2]. Whereas several PAMPs of other gram-negative bacteria, such as LPS, flagellin or hypomethylated CpG have been shown to activate NF-κB by binding to toll-like receptors dedicated to their detection, none of these innate detection mechanisms appeared to be operative in the context of an *H. pylori* infection [3]. The LPS of *H. pylori* is modified to avoid binding to TLR4, its flagellin is mutated in the TLR5 binding site, and its DNA has anti- rather than pro-inflammatory properties [4-7]. The molecular basis underlying NF-κB activation by *H. pylori* remained enigmatic until the recent discovery, published independently by three labs [8-10], of the activation of the α-kinase 1 (ALPK1) and TRAF-interacting protein with forkhead-associated domain (TIFA) axis by inner core LPS biosynthetic intermediates such as d-glycero-β-d-manno-heptose-1,7-bisphosphate (HBP) or ADP-l-glycero-β-d-manno-heptose (ADP-heptose) (Figure 1). The ALPK1/TIFA pathway was first identified to be activated by *Neisseria* species [11], but was later confirmed also for other gram-negative pathogens such as *Shigella* [12] and *Yersinia* [13]. In the case of *H. pylori*, mutants that lack the bifunctional enzyme HldE (RfaE) and therefore cannot synthesize either HBP or ADP-heptose fail to activate ALPK1/TIFA signaling, and gastric epithelial cells that have been engineered to lack ALPK1 or TIFA cannot activate NF-κB in response to *H. pylori* [8-10]. ADP-heptose appears to be the more important of the two proposed ALPK1 ligands, as it binds to ALPK1 with greater affinity and is present in much larger quantities in *H. pylori* extract [13,14]. Specifically, a recent publication found β-ADP-heptose to be present in *H. pylori* at >10 times higher concentrations than HBP and to be 100-fold more potent than HBP in activating ALPK1/TIFA–controlled innate immune responses in epithelial cells [14]. ADP-heptose is active when added to cells extracellularly, whereas HBP acts only when delivered by lipofection or electroporation [14]. The binding of ADP-heptose, and possibly of HBP, to ALPK1 stimulates its kinase domain to phosphorylate and activate TIFA [13], which forms large complexes (TIFAsomes) that also include interactors such as TRAF2 [9] (Figure 1). Such activation of the upstream receptors leads to the phosphorylation of the inhibitor of NF-κB, IκB-α, followed by its polyubiquitination and proteasomal degradation, which in turn releases the NF-κB heterodimer p65/p50, normally sequestered in the cytosol, for nuclear translocation and activation of target genes (Figure 1). NF-κB signaling has often been described as the lynchpin linking inflammation and cancer, in particular in settings of inflammation-associated

cancers of the colon and the liver [15,16]. NF-κB has tumor cell-extrinsic and -intrinsic oncogenic properties [17], promoting the production of reactive oxygen and nitrogen species and inducing DNA damage and oncogenic mutations on the one hand [18] and promoting tumor cell survival on the other [19,20]. NF-κB activation can also compromise genomic instability directly, as DNA double strand break formation upon *H. pylori* infection is linked to NF-kB activation through a functional T4SS [21]. Given the relevance of this signaling pathway not only for pro-inflammatory immune responses to *H. pylori*, but presumably also in the context of gastric carcinogenesis, the recent description of the upstream mediators of NF-κB activation during *H. pylori* infection represents an important milestone indeed.

H. pylori interacts with various subsets of myeloid cells in the gastric lamina propria that have diverse and opposing functionalities

One of the consequences of NF-κB activation in gastric epithelial cells is the production of a gradient of the chemokine CCL2 (also called MCP-1, macrophage chemotactic protein-1), which serves as a central chemokine attracting myeloid cells to infected tissues. Mice lacking expression of the CCL2 receptor CCR2 are incapable of responding properly to *H. pylori* infection [22]. These mice, in contrast to their wild type counterparts, fail to recruit at least six immunophenotypically distinct subsets of monocytes, macrophages and dendritic cells (DCs) to the infected gastric mucosa; in several myeloid subsets (monocytes, macrophages and $CD11b^+$ DCs), this defective recruitment is cell-intrinsic, i.e. directly due to defective CCL2/CCR2 signaling in the respective cells [22]. As a consequence of their defect in recruiting myeloid cells, $CCR2^{-/-}$ mice cannot mount proper T-helper-1 (Th1) responses to *H. pylori* and are strongly hypercolonized. Most studies to date have examined the interactions of *H. pylori* with macrophages and DCs *in vitro*, and have largely come to the conclusion that the ability of *in vitro*-derived myeloid cells to present antigen, undergo maturation and prime T-cell responses is compromised in the presence of *H. pylori* [6,23-25]. This ability of *H. pylori* to induce tolerogenic over immunogenic responses has been demonstrated for human monocyte-derived dendritic cells, which fail to secrete IL-6 and other pro-inflammatory cytokines upon exposure to *H. pylori*, and gain the ability to prime the differentiation of naïve T-cells into $Foxp3^+$ regulatory T-cells (Tregs), but not Th1 or Th17-polarized effector T-cells [23,24], and also for murine DCs, which are re-programmed in a similar manner [26]. Early work in mouse models from multiple labs has shown that DCs in the *H. pylori*-infected gastric lamina propria of mice are semi-mature (i.e. express MHCII, but only very low amounts of co-stimulatory molecules and pro-inflammatory cytokines) and skewed to promote Treg

differentiation [26,27], and that their depletion, or the depletion of Tregs, promotes *H. pylori* immune control and clearance [26-28]. More recent work suggests that the myeloid network that *H. pylori* encounters in the gastric mucosa is highly complex, and consists of at least six distinct myeloid populations, presumably with quite diverse functions [22]. Of these, three are considered bona fide DCs, as they express CD11c and depend on the growth factor FLT3 ligand for their differentiation from bone marrow precursors; the others are macrophages and monocytes expressing the respective lineage markers F4/80, CD64 and Ly6C. The use of RFP^+ bacteria has demonstrated that all macrophages and monocytes, and some, but not all DC lineages come in direct contact with live bacteria in the gastric lamina propria [28]. The lack of appropriate mouse models missing specific populations of macrophages or DCs has hampered investigations into the division of labor among these cells, and their specific functionalities in the context of bacterial infections in general and *H. pylori* infection in particular. One recent step forward has been a detailed investigation of the role of $CD103^+CD11b^-$ DCs in *H. pylori* infection, which was facilitated by the availability of a mouse strain specifically lacking this DC lineage, but no other myeloid populations [29]. $CD103^+CD11b^-$ DCs rely on the transcription factor BATF3 (basic leucine zipper transcriptional factor ATF-like 3) for their differentiation from a common DC progenitor [30] and therefore fail to develop in $BATF3^{-/-}$ mice; the lineage is best known for its critical role in antiviral defense and in cross-presentation of antigens [31]. $BATF3^{-/-}$ are incapable of controlling an experimental *H. pylori* infection, which could be attributed to their inability to launch proper Th1 responses [29] (Figure 2). A similar defect in Th1 immunity was reported in a tumor model and another bacterial infection model using *Mycobacterium bovis* BCG [29]. More detailed mechanistic studies showed that, while T-cell priming and Th1 differentiation in the draining lymph nodes was not impaired due to BATF3 deficiency, these cells lacked expression of the surface receptor CXCR3 and therefore failed to home to infected tissues in response to gradients of the chemokines and CXCR3 ligands CXCL9, CXCL10 and CXCL11 (Figure 2). The same problem applied to regulatory T-cells, which differentiated normally, but failed to upregulate CXCR3 in the absence of $CD103^+CD11b^-$ DCs [29] (Figure 2). The dual homing defect of both Tregs and Th1 cells explains why $BATF3^{-/-}$ mice exhibit phenotypes in models of immunity [32-34] and immune tolerance [35-37]. DC lineages other than the above-mentioned $CD103^+CD11b^-$ DCs have been less accessible to experimental studies, as mouse strains with specific defects in $CD103^+CD11b^+$ or $CD11b^+CD103^-$ DCs are not readily available. Circumstantial evidence suggests that the loss of $CD11b^+$ $CD103^-$ DCs has the opposite effect as the loss of BATF3-dependent cells on *H. pylori* immune control and colonization. In $NLRP3^{-/-}$ mice, which lack $CD11b^+$ $CD103^-$ DCs in all mucosal tissues in addition to their general defect in NLRP3 inflammasome activation, *H. pylori* is controlled more effectively due to stronger, dysregulated Th1 responses [22]. More sophisticated studies are needed to confirm that $CD11b^+$ DCs

indeed have tolerogenic activities that counter-regulate the functions of BATF3-dependent DCs at the *H. pylori*/host interface.

H. pylori deploys two immunomodulatory molecules, the γ-glutamyl-transpeptidase and the vacuolating cytotoxin, to reprogram DCs and promote persistent infection

H. pylori has evolved at least two independent mechanisms to ensure the tolerogenic reprogramming of DCs and to thereby promote its persistence in the human host. Both have been studied in quite some detail *in vitro* and *in vivo*, and the genetic determinants encoding the two factors are present in all *H. pylori* isolates sequenced so far. One of the two is a secreted enzyme with γ-glutamyl-transpeptidase activity (GGT) that is highly conserved within *Helicobacter* strains and contributes to bacterial colonization *in vivo* [38-40]. GGT catalyzes the hydrolysis and transpeptidation of γ-glutamyl compounds such as oxidized and reduced glutathione, and also hydrolyzes glutamine to glutamate and ammonia [41]. Both glutamate and glutamine have well-documented immunomodulatory and tissue-protective properties in settings of gut inflammation and autoimmunity, as well as in gastritis caused by *H. pylori* infection [42-44]. In the context of *H. pylori*'s interaction with DCs, glutamate produced due to GGT enzymatic activity activates glutamate receptors on the surface of DCs, and tolerizes DCs by inhibiting cAMP signalling [24]. The tolerogenic state of human DCs exposed to GGT-proficient, but not -deficient bacteria was evidenced by reduced IL-6 production, and the preferential differentiation of co-cultured naïve T-cells into $Foxp3^+$ Tregs [24]. Similar results were reported for murine DCs, which also drive Treg differentiation as a function of GGT exposure *in vitro* and *in vivo* [45]. DCs isolated from the mesenteric lymph nodes of mice infected with WT *H. pylori* induced Foxp3 expression in T-cells, whereas DCs isolated from mice infected with GGT-deficient bacteria did not [45]. Whether GGT acts via glutamate production or some other product of its enzymatic activity to tolerize DCs *in vivo* remains unknown; however, the inability of ΔGGT *H. pylori* to efficiently colonize experimentally infected mice and to suppress anti-*Helicobacter* T-cell responses suggests that GGT is an essential immunomodulator also *in vivo* [40,45].

The second immunomodulatory molecule expressed by all *H. pylori* strains, the vacuolating cytotoxin VacA, is also secreted, essential for high level colonization and known to target DCs [45-47]. VacA can be traced to at least two different myeloid populations using flow cytometry-based tracking strategies, i.e. to $CX3CR1^+F4/80^+$ macrophages and to $CD11b^+CD103^-CX3CR1^{dim}$ DCs in the gastric lamina propria, and to their cellular counterparts in the Peyer's patches [46] (Figure 3). Macrophages sorted from the gastric lamina propria of *H. pylori*-infected mice showed enhanced IL-10 and TGF-β expression that

was dependent on VacA proficiency of the infecting strain; in DCs, the expression of IL-23, a cytokine driving protective Th17 responses, was downregulated by *H. pylori* in a VacA-dependent manner [46] (Figure 3). The tolerogenic microenvironment created by VacA, presumably through its activities on myeloid cells, promotes the preferential induction of Treg over effector T-cell responses [46]. The peripherally induced, $Foxp3^+$ $neuropilin^-$ $ROR\gamma t^+$ Tregs that are a hallmark of *H. pylori* infection of the stomach, especially of mice infected early in life, surprisingly also home to the lungs, where they are thought to suppress T-cell responses to allergens (Figure 3). Many of the effects of VacA on myeloid cells observed *in vivo* can be recapitulated by simple bone marrow-derived (BM) macrophage or DC cell culture models. BM-macrophages express IL-10 upon exposure to VacA-proficient bacteria or purified VacA [46]. Indeed, the STAT3 phosphorylation and activation that is downstream of (autocrine or paracrine) IL-10 receptor signaling is known to be required for tolerogenic programming of DCs, as neutralization of IL-10, or the genetic ablation of STAT3 in DCs, both compromise the ability of *H. pylori* to inhibit DC maturation [25], favoring immune tolerance and chronic infection.

VacA has also recently been reported to have another, much more direct effect on *H. pylori* persistence in its host [48]. This function is restricted to specific alleles of the *vacA* gene that encode a version of VacA that has vacuolating (hence the name) and cytotoxic activity in gastric epithelial cells and interferes with endolysosomal trafficking [48]. The *vacA* gene exhibits genetic polymorphism, with allelic variants differing in the signal (s1/s2), middle (m1/m2) and intermediate (i1/i2) regions of the gene [49]. These variants differ in their cytotoxicity, with s1i1 being toxigenic and strongly associated with gastric cancer, and s2i2 being considered non-toxigenic [49]. Whereas a mouse-colonizing strain of *H. pylori* that was engineered to express s1i1 VacA was found to be present within parietal cells (i.e. the acid-producing cells of the corpus region of the stomach) and specifically within vacuoles in these cells, this was not true for an isogenic strain expressing s2i2 VacA [48]. This activity of s1i1 VacA could be attributed to it targeting the lysosomal calcium channel TRPML1, which disrupts endolysosomal trafficking and drives the formation of vacuoles allowing for intracellular residence of *H. pylori* [48]. In *trpml1*-null mice, even *H. pylori* that lacked toxigenic VacA was found to colonize enlarged dysfunctional lysosomes. The VacA-driven intracellular lifestyle of *H. pylori* is believed to protect the bacteria from unfavorable conditions such as antibiotic exposure, and to provide a reservoir allowing for recolonization of the stomach once conditions have improved. Although intracellular bacteria are rare relative to the mucus- and mucosa-associated population, the phenomenon has also been reported for human biopsies by other groups [50,51], with reported intracellular rates of around 1% of all bacteria present. The combined results, and newly identified link to VacA, provide a plausible explanation for the extraordinary decade-long persistence of *H. pylori* and its relative resistance to (especially single agent) antibiotic eradication therapy.

H. pylori interacts with eosinophils in the gastric lamina propria, but evades eosinophil bactericidal activities

Whereas the interactions of *H. pylori* with DCs and macrophages are fairly well understood and have received much attention (see above), little is known to date on *H. pylori*'s interaction with eosinophils, a type of granulocyte that arises in the bone marrow from granulocyte/monocyte progenitors and represents one of the numerically dominant leukocyte populations of the gastrointestinal tract at steady state. In a recent analysis of eosinophils and the roles they play in the context of *H. pylori* infection, this cell type was found to be recruited in large numbers to the infected gastric mucosa, to interact directly with live (RFP^+) bacteria, and to become activated as a consequence [52]. The depletion of eosinophils, either through neutralization of IL-5, a cytokine that serves as growth and differentiation factor for eosinophils, or by genetic ablation, resulted in improved clearance of *H. pylori* through an enhanced Th1 response [52] (Figure 4). The proposed mechanism of immune regulation by eosinophils involves upregulation of PD-L1 on eosinophils upon *H. pylori* contact, especially in the simultaneous presence of IFN-γ, which in turn suppresses the activities of PD-1-positive $CD4^+$ T-cells through direct cell-to-cell interactions (Figure 4). Parallel mechanisms appear to also be operative in other parts of the gastrointestinal tract, where the loss of eosinophils also results in excessive T-cell responses to the normal microbiota [52]. Eosinophils are better known for their anti-parasitic activities, but also help clear out certain bacterial infections [53]. Interestingly, *H. pylori* is unresponsive to the bactericidal activities of eosinophils, whereas other mucosal GI tract pathogens (e.g. *Citrobacter rodentium*) are readily killed by eosinophils *in vitro* and *in vivo* [52]. Bacterial killing requires eosinophil extracellular traps (EETs), which are decorated with bactericidal products such as eosinophil cationic protein and major basis protein and which are deployed upon contact with *Citrobacter*, but not *Helicobacter* [52]. Early work has shown that eosinophils are recruited to the gastric mucosa of *H. pylori*-infected humans [54]; human eosinophils developing in humanized mice that have been reconstituted with a fully human immune system interact with and become activated upon contact with *H. pylori* [52]. The combined results suggest that *H. pylori* has evolved to not only avoid killing by this important effector cell of the innate immune system, but to also reprogram it to suppress T-cells and promote persistence.

Systemic manifestations of *H. pylori* and its influence on co-occurring parasitic infections

The gastric mucosa represents the only reservoir for *H. pylori* in the human host. Nevertheless, numerous studies conducted in experimentally infected animals and large cohorts of infected humans now point to systemic consequences of localized *H. pylori* infection on immune responses to allergens, autoantigens, parasites and bacteria. For example, it is now very well established that *H. pylori* infection, especially in children and young adults, reduces allergen-specific immune responses, diminishes the risk of allergic asthma and other allergic disease manifestations, and alleviates allergy severity in high risk populations, both rural and urban, in various geographical areas of the world [55-58]. In the most recent, and one of the most comprehensive studies conducted so far, 856 Ethiopian children were followed from birth to 6 years of age and examined longitudinally for the appearance of atopy and allergic manifestations of all types relative to their *H. pylori* infection status [59]. This study, like many similar ones conducted previously, among them a recent study out of Egypt (180 children) [60] and a meta-analysis of 24 independent studies [61], confirmed that colonization with *H. pylori* is inversely related with atopy risk, data that are consistent with the hypothesis that early exposure to *H. pylori* is particularly beneficial in this context. Experimental studies in mouse models confirm a protective effect of neonatal *H. pylori* infection on the severity of allergic airway inflammation (a mouse model of allergic asthma) and of food allergy [62-64], and link the benefits of harboring *H. pylori* to its VacA and the regulatory T-cells that are induced in a VacA-dependent manner [45,46] (Figure 3). The first data are now available that support a role of Tregs also in allergy protection of *H. pylori*-infected humans. Specifically, when analyzing Th1 and Treg responses of 49 infected and 58 uninfected adult donors, Karen Robinson and associates found significantly higher frequencies of IL-10-secreting $CD4^{+}CD25^{hi}$ Tregs, but not of *H. pylori*-specific Th1 cells to be present in peripheral blood of infected relative to uninfected donors [65]. Total and allergen-specific IgE concentrations were lower when there was a strong Treg response and blocking IL-10 *in vitro* restored IgE responses to allergens. The beneficial effects of *H. pylori* were particularly pronounced with $CagA^{+}$ strains or those expressing the more active i1 form of VacA [65]. The peripheral blood data complement earlier work, also conducted on large human populations (84 *H. pylori*-infected and 46 uninfected patients), showing that Tregs migrate to, and accumulate in the *H. pylori*-infected stomach [66].

There is less abundant literature on a possible inverse link between *H. pylori* and autoimmunity. One study found *H. pylori* seroprevalence of multiple sclerosis patients to be roughly half that of healthy controls, and symptoms of experimental autoimmune encephalitis (the gold standard mouse model of multiple sclerosis) to be alleviated as a consequence of experimental *H. pylori* infection [67]. Chronic inflammatory disorders of the lower bowel in contrast, especially the inflammatory bowel diseases ulcerative colitis and Crohn's disease, are very strongly inversely correlated with *H. pylori* infection, with extremely low *H. pylori* seroprevalence among patients presenting with IBDs [68]. Mouse model

data support the notion that *H. pylori* is protective against IBD in settings of DSS-induced or T-cell transfer-induced colitis [69].

In light of the strong modulation of allergen-specific immune responses and chronic inflammatory conditions, it is not far-fetched to assume that the immune control of parasites and of acute or chronic bacterial infections is similarly affected by the presence of *H. pylori*. There is a surprising paucity of literature on this topic. A recent study was the first in a long time to address how infection with a parasite, *Schistosoma mansoni* would affect *Helicobacter* immune control and vice versa [70]. To mimic the most likely sequence of events in humans, mice were first infected with *H. pylori*, followed by *S. mansoni* five weeks later. Both infectious agents had robust effects on one another: *H. pylori* colonization levels were increased in the simultaneous presence of *S. mansoni*, and local Th1 responses were concomitantly decreased resulting in generally lower inflammation. Rather than homing to the infected stomach, Th1 cells appeared to be redirected to the (*S. mansoni*-colonized) liver, to which they followed a gradient of Th1-recruiting cytokines such as CXCL9, 10 and 11 [70]. Other T-cell subsets did not appear in the liver, indicating that the redirection is highly Th1-specific. The phenomenon was restricted to the early, Th1-polarized phase of *S. mansoni* infection, whereas gastric parameters normalized during the later, Th2-polarized phase. The trend towards lower gastritis in the presence of parasitic infection has been reported before by others in a study where concomitant infection of *Helicobacter felis* with *Heligmosomoides polygyrus*, a murine nematode with an enteric life cycle, reduced *H. felis*-specific gastritis and gastric preneoplastic pathology [71]; the authors interpreted these earlier findings by postulating that Th2 cytokines expressed during helminth infection antagonized pro-inflammatory and immunopathological Th1 responses, thereby reducing gastric pathology. In both scenarios, colonization levels of the helminths, *S. mansoni* and *Heligmosomoides polygyrus* remained more or less unchanged; however, liver damage due to *S. mansoni* was reduced, which manifested in smaller liver granulomas, lower alanine aminotransferase (ALT) levels (a measure of liver cell destruction or disease) in the serum and lower collagen levels in the liver [70]. The protective effects of *H. pylori* infection on the liver were attributed to higher IL-10 levels, stronger hepatocyte-protective IL-10-dependent STAT3 activation and higher levels of a decoy receptor for the pro-fibrotic Th2 cytokine IL-13 in co-infected mice [70]. As co-infection of helminths and *H. pylori* must be considered the norm rather than an exception in many parts of the world (schistosomiasis is still endemic in 78 countries according to the WHO), this type of work, albeit experimentally challenging, is highly clinically relevant. The same is probably true for concomitant bacterial infections, on which virtually no information is currently available. Intriguing data from *Helicobacter hepaticus* infection models, which are also characterized by strong and persistent Treg responses [72], suggest that *H. hepaticus* prevents proper immune control of *Mycobacterium*

tuberculosis and enhances lung pathology [73]. Similar immunomodulatory mechanisms might also compromise *Mycobacterium tuberculosis* immune control in the presence of *H. pylori*.

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Figure legends

Figure 1. Type IV secretion system-expressing *Helicobacter pylori* is detected by gastric epithelial cells through binding of the LPS precursor β-ADP-heptose to ALPK1, resulting in TIFAsome formation and NF-κB activation. *H. pylori* intermediates of the LPS inner core biosynthetic pathway, i.e. β-ADP-heptose and possibly also HBP, are released by T4SS-positive *H. pylori* and enter the cytoplasm of target cells, where they bind to alpha-kinase 1 (ALPK1) leading to its activation. ALPK1 subsequently phosphorylates TIFA, resulting in its multimerization and TIFAsome formation. TIFAsomes activate NF-κB through a signaling cascade involving TRAF2 and TAK and the phosphorylation and subsequent proteasomal degradation of the inhibitor of NF-κB, IκBα. NF-κB target genes include numerous cytokines and chemokines (the best-studied in the *H. pylori* context being IL-8), surface markers, other pro-inflammatory molecules, and survival factors.

Figure 2. BATF3-dependent DCs drive immune control of *H. pylori* by producing chemokines and priming Th cells to express CXCR3. In the gastric mucosa of wild type mice, BATF3-dependent DCs and several other myeloid lineages sample *H. pylori* and trigger a vigorous mixed Th1/Th17 response, which nevertheless is incapable of completely clearing *H. pylori*. *H. pylori*-specific Th responses are primed in the draining mesenteric lymph nodes. Th1 cells, but not Th17 cells, home to infected tissue following a gradient of CXCL-9, -10, -11 and probably other chemokines. In the absence of BATF3-dependent DCs, Th1 differentiation (evidenced by Tbet expression and IFN-γ production) occurs normally; however, Th1 cells primed in the absence of this lineage fail to upregulate the chemokine receptor CXCR3 and therefore fail to traffic to the *H. pylori*-infected gastric mucosa. The same

mechanism also explains the deficiency of BATF3$^{-/-}$ mice in controlling other bacterial infections and also tumors, and further accounts for defective *H. pylori*-specific Treg responses in BATF3$^{-/-}$ mice.

Figure 3. *H. pylori* VacA promotes chronic infection by tolerizing myeloid cells and promoting peripheral Treg differentiation. *H. pylori* releases at least two immunomodulatory molecules in its gastric mucosal niche. One of them, the vacuolating cytotoxin VacA, interacts with various populations of myeloid cells, for example CD11b^{+} DCs and macrophages, resulting in their tolerogenic programming. VacA-experienced antigen-presenting cells migrate to the draining lymph nodes, where they promote the differentiation of naïve T-cells into regulatory T-cells that co-express Foxp3 and RORγt, but are negative for neuropilin, i.e. so-called peripherally induced pTregs. pTregs traffic to the infected stomach, but are also found at higher frequencies than normal in the lungs of infected mice, especially if exposure to *H. pylori* occurs early in life. pTregs in the lung are believed to suppress allergen-specific T-cell responses, explaining why *H. pylori*-infected children and young adults are at lower risk of developing allergic asthma than naïve individuals.

Figure 4. *H. pylori* interacts with eosinophils in the gastric mucosa, but has evolved to evade the bactericidal activities of this cell type. Eosinophils are recruited to the *H. pylori*-infected gastric mucosa in large numbers, especially during the chronic phase (i.e. six weeks post infection and later) of the infection. Eosinophils directly encounter *H. pylori* as evidenced in experiments with red fluorescent protein (RFP)-expressing bacteria. Eosinophils in the infected mucosa upregulate PD-L1 in settings of high IFN-γ production, which enables them to efficiently inhibit Th1 cell expansion and activity. As a consequence, eosinophil-proficient mice are colonized at higher levels than mice in which eosinophils have been depleted either by genetic ablation or by treatment with an IL-5-neutralizing antibody. Eosinophils in other bacterial infection settings exhibit bactericidal properties that depend on their ability to deploy extracellular traps (EETs). *H. pylori* has evolved to avoid triggering EETs, and therefore cannot be killed by eosinophils.

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One of three studies describing the prerequisites of ALPK1/TIFA activation upon *H. pylori* infection

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Figures

Figure 1

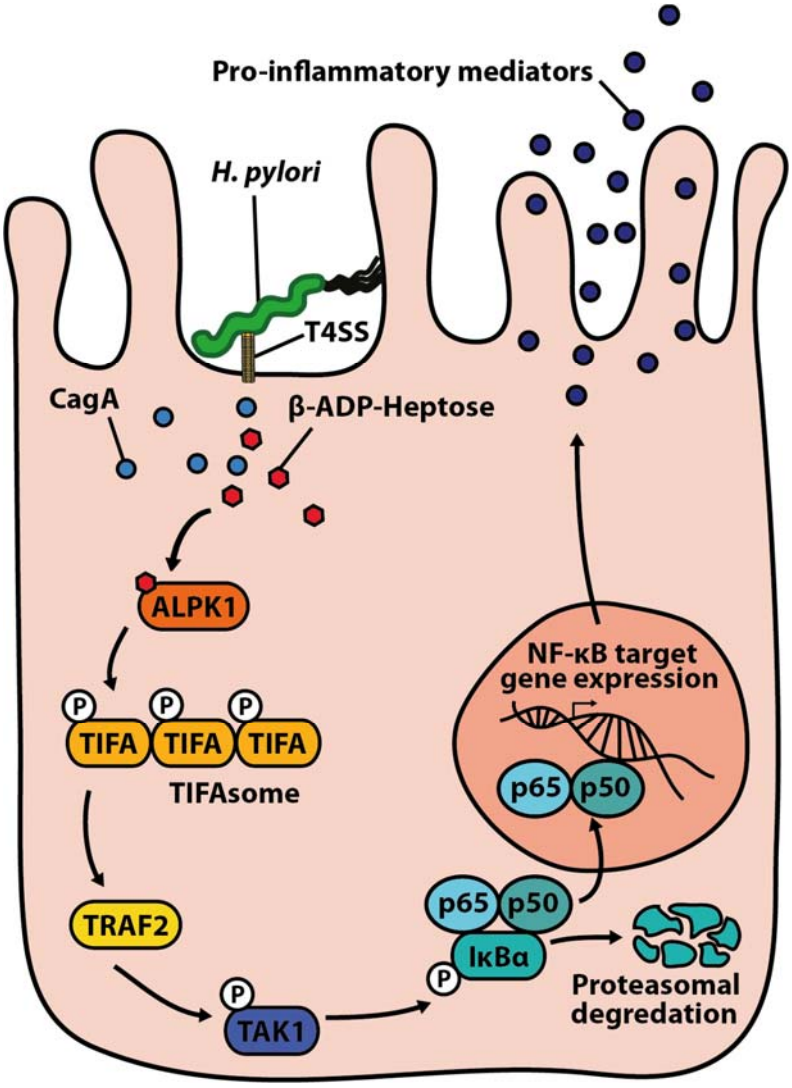


Figure 2

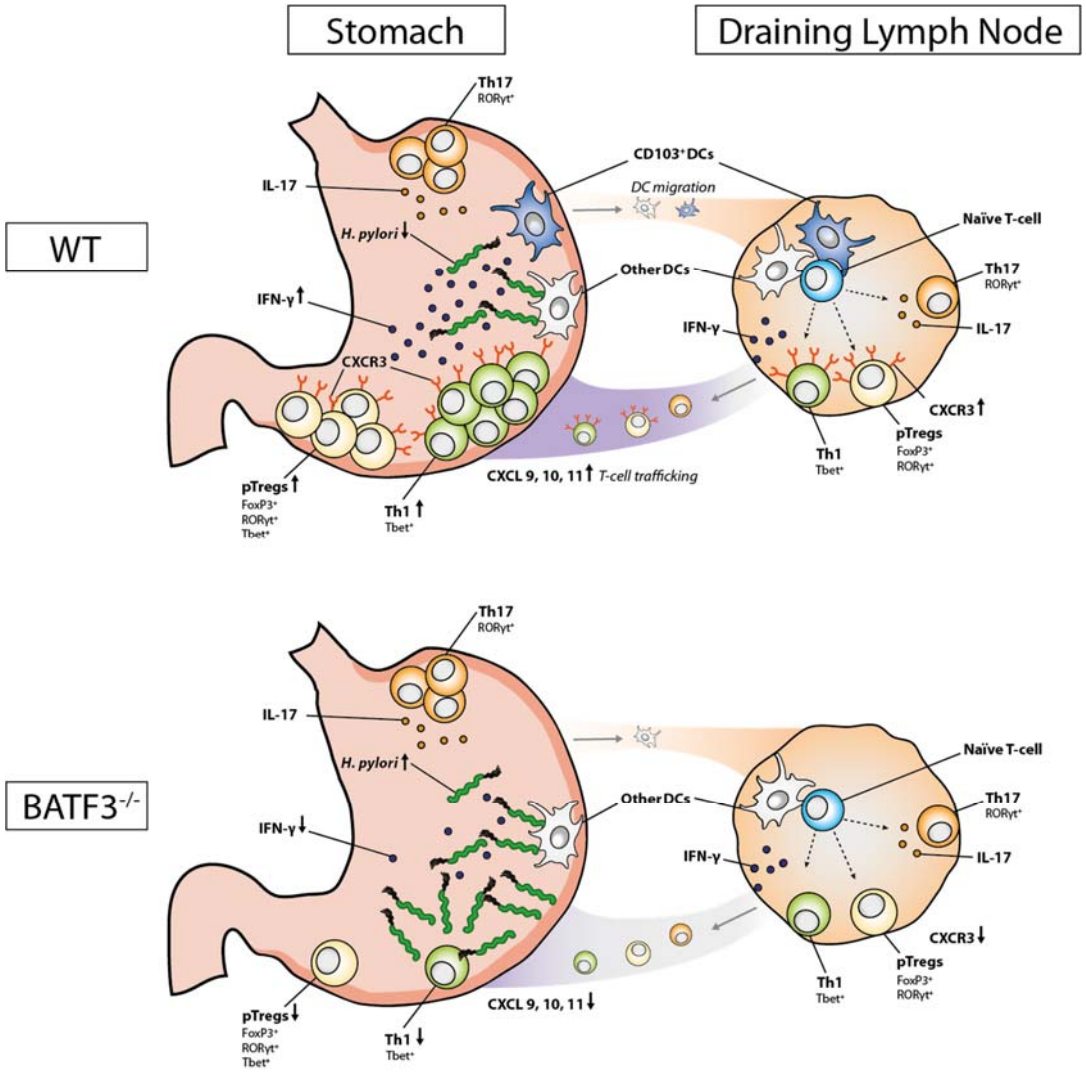

Stomach
Draining Lymph Node
WT
BATF3-/-
Th17
RORγt+
IL-17
H. pylori
IFN-γ
CXCR3
pTregs
FoxP3+
RORγt+
Tbet+
Th1
Tbet+
CD103+ DCs
DC migration
Other DCs
Naïve T-cell
CXCL 9, 10, 11
T-cell trafficking

Figure 3

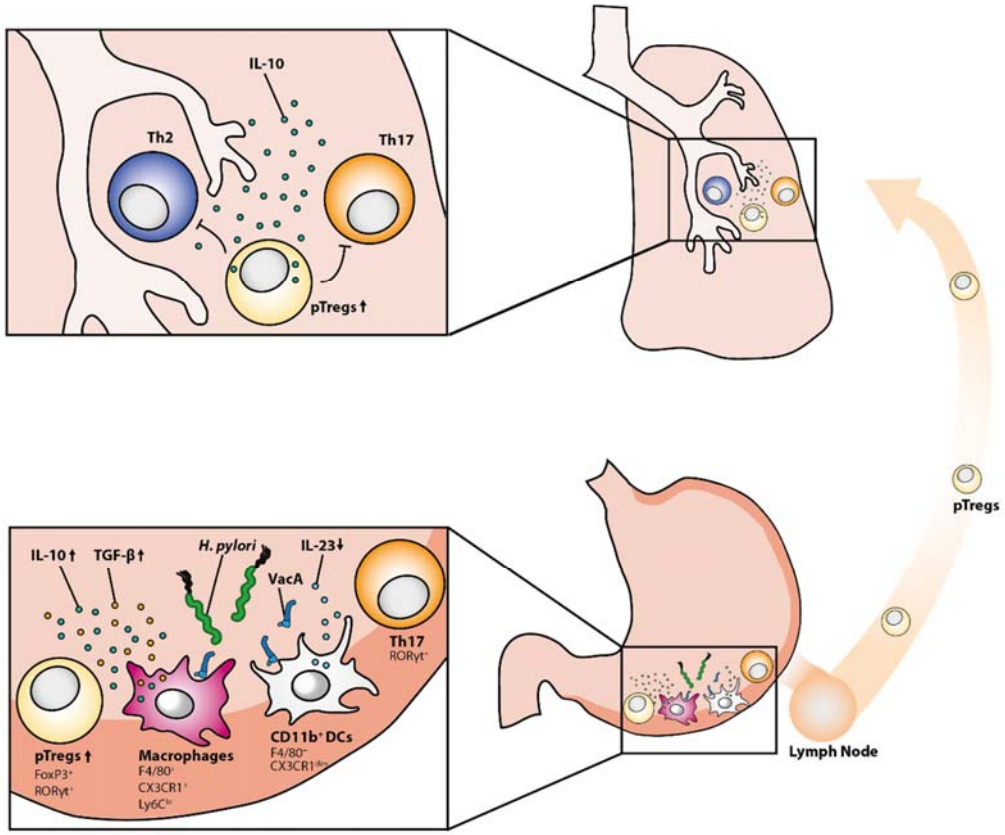
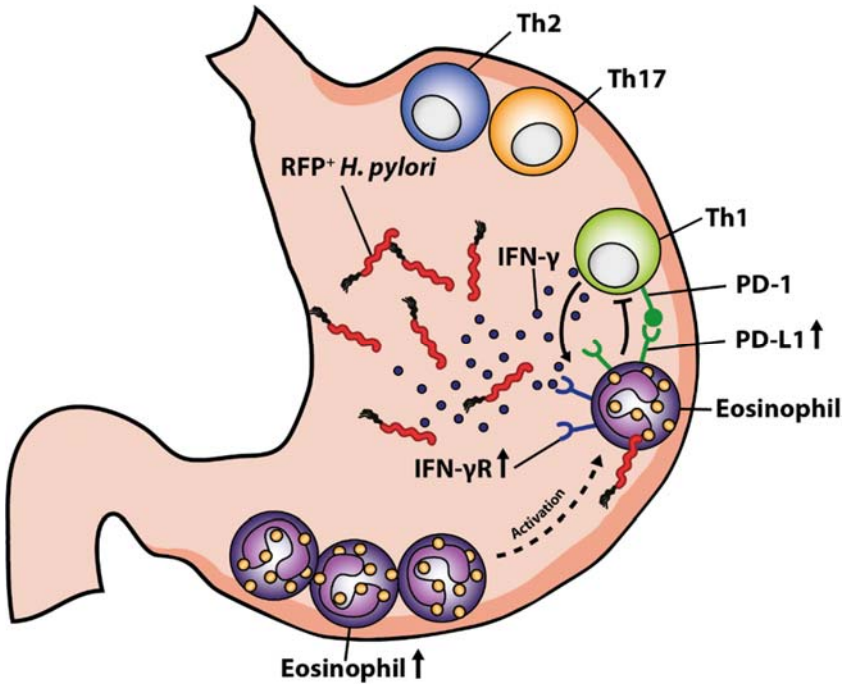

IL-10
Th2
Th17
pTregs ↑
pTregs
IL-10 ↑
TGF-β ↑
H. pylori
IL-23 ↓
VacA
Th17
RORγt+
pTregs ↑
FoxP3+
RORγt+
Macrophages
F4/80+
CX3CR1+
Ly6Clo
CD11b+ DCs
F4/80−
CX3CR1hi
Lymph Node

Figure 4


Th2
Th17
RFP+ H. pylori
Th1
IFN-γ
PD-1
PD-L1
Eosinophil
IFN-γR
Activation
Eosinophil